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SOME PROPERTIES OF THE CURLY-TOP VIRUS[1]

HENRY H. P. SEVERIN[2] AND JULIUS H. FREITAG[3]

(Contribution from the Division of Entomology and Parasitology, College of Agriculture, University of California, cooperating with the United States Department of Agriculture, Bureau of Entomology.)

INTRODUCTION

According to some plant pathologists the virus diseases of plants are divided into two groups, the mosaic and the yellows diseases. The mosaic diseases under optimum conditions cause a mottling of the leaves, usually in the growing tissues of the plants. Some of the mosaic diseases are highly infectious, while others are not, and some have not been transmitted mechanically. Some of the mosaic viruses have been shown to be filterable, but others are not. Intracellular, cell inclusions, or x-bodies are associated with many diseases of this group. Mosaic viruses are transmitted mostly by sucking insects, usually aphids, rarely by chewing insects. Some single species of aphids are associated with the dissemination of many separate mosaic viruses, other species with the spread of but one mosaic virus. The peach aphid *(Myzus persicae)* is associated with the dissemination of fourteen separate virus diseases, and the potato aphid *(Macrosiphum solanifolii)* is associated with the spread of six plant viruses.(57) Some insects are mechanical vectors of mosaic viruses, as in the case of cucumber mosaic,(15) and retain the virus for only a short time. The aphid vector of spinach blight retains the infective power for a period of five days.(38) Some mosaic diseases are transmitted through the seeds.

Kunkel(36) lists 24 virus diseases of plants outside of the mosaic group, including well-known destructive yellows diseases in California, such as curly top of sugar beets; yellows of china aster, celery, and lettuce;

[1] Received for publication February 28, 1933.

[2] Associate Entomologist in the Experiment Station.

[3] Research Assistant in the Experiment Station.

strawberry yellows; and leafroll of potato. "In the yellows type of disease, chlorosis is general throughout the affected parts."[36] Most of the virus diseases in this group have not been transmitted mechanically, except by budding or grafting, and hence plant pathologists have given less attention to the filterability of these viruses. Cell inclusions or x and y-bodies have been reported in this group of diseases. In some of these diseases a specific relation is believed to exist between a particular virus and its insect vector. Some of the viruses of these diseases are carried by a single species of leafhopper or aphid. In the case of potato leafroll, however, the virus is transmitted by six species of aphids, two species of leafhoppers, a flea beetle *(Psylliodes affinis),* and the larva of *Tipula paludosa.*[57] An incubation period occurs in some of the insect vectors, such as *Eutettix tenellus,*[7, 48, 53, 60] which transmits curly top to a large number of host plants;[20, 50, 51, 52, 54] *Cicadula divisa (= C. sexnotata* Aucct.) which carries yellows to a large number of ornamental flowering plants;[35, 37] *Cicadulina mbila*[59] which transmits streak disease to corn; and *Myzus persicae*[18, 57] which transmits leafroll to potato. It has been demonstrated, however, that *Eutettix tenellus* transmits curly top in short periods, on rare occasions, probably by contamination of mouth parts.[53] Many insect vectors retain the infective power for long periods, sometimes throughout the life of the insect, and this has been assumed as evidence of a multiplication of the virus in the body of the insect. No disease in this group has been reported as passing through the seeds.

Chemical investigations by Dunlap[17] and other scientists on the two classes of virus diseases seem to indicate that in the mosaics there is a higher nitrogen and lower carbohydrate content than in healthy leaves, while in the yellows group there is a lower nitrogen and higher carbohydrate content.

The filterability of the curly-top virus from both diseased sugar beets and infective beet leafhoppers has been demonstrated.[55]

The investigations reported in this paper were undertaken on some other properties of the virus to determine whether or not it has characteristics which might further differentiate the yellows group from the mosaics. By transmission experiments with previously noninfective beet leafhoppers a study was made of the virus in the juices from various parts of diseased beets, the effect of aging on the virus under aerobic and anaerobic conditions, cultivation of the virus outside of living plants, resistance to drying in plant tissues and infective beet leafhoppers, inactivation of virus with juices from an immune host plant, purification, dilution, thermal death point, and freezing of the virus.

GENERAL METHODS

Beet Extracts.—In the preparation of juice from the blades or petioles from diseased beets, the leaves were washed in distilled water, and ground to a pulp in a sterilized food chopper. In experiments reported in this paper the "beet root," that is, the sugar beet with the crown and lateral and tap roots removed, was used. The beet roots were scrubbed with a brush, sliced, and ground in a food chopper. The pulp was then placed in several layers of cheesecloth and the juice pressed out into a sterile pan by hand.

In earlier experiments[55] sterile beet-root juice was prepared by placing healthy beet roots, cut in small pieces, in an autoclave for a period of at least an hour, and the juice was extracted with steam pressure varying from 18 to 20 pounds. The preparation of sterile beet-root juice was simplified in later experiments. The juice was centrifuged for an hour, autoclaved, passed through filter paper, and again autoclaved.

Extract from Crushed Infective Beet Leafhoppers.—In the preparation of the virus extract from infective beet leafhoppers, the insects were captured with a pipette (fig. 1) and put in small vials containing beet-root juice and beet-sugar solution. The bottles were shaken so that the wings became wet and the insects were unable to fly. One gram of infective leafhoppers, or approximately 1,000 specimens, which had completed the nymphal stages on diseased beets, were used in experiments reported in this paper. The insects were transferred from the vials to a mortar by repeatedly filling a medicine dropper with the solution and forcing a stream of the liquid into the vial and then dumping the contents of the vial into the mortar. The liquid was removed from the mortar and the insects were ground with pulverized pyrex glass with a pestle in a Schultz mechanical grinder (fig. 2). To each gram of crushed leafhoppers, 99 cc of a mixture of equal parts of steam-extracted or autoclaved beet-root juice and a 5 per cent beet-sugar solution were added.

Centrifugation.—The period of centrifugation of diseased beet juice and crushed infective beet leafhoppers was usually one hour. When 1,000 to 2,000 cubic centimeters of beet juice was required, the centrifugation speed was 2,000 revolutions per minute, and with smaller quantities of juice the speed was 3,500 revolutions per minute. When the Sharples supercentrifuge was used at a speed of 40,000 revolutions per minute, the juice from the blades, petioles, blades and petioles combined, or beet root was first centrifuged at 2,000 revolutions per minute to throw down fragments of tissues that would otherwise clog the feed nozzle of the supercentrifuge.

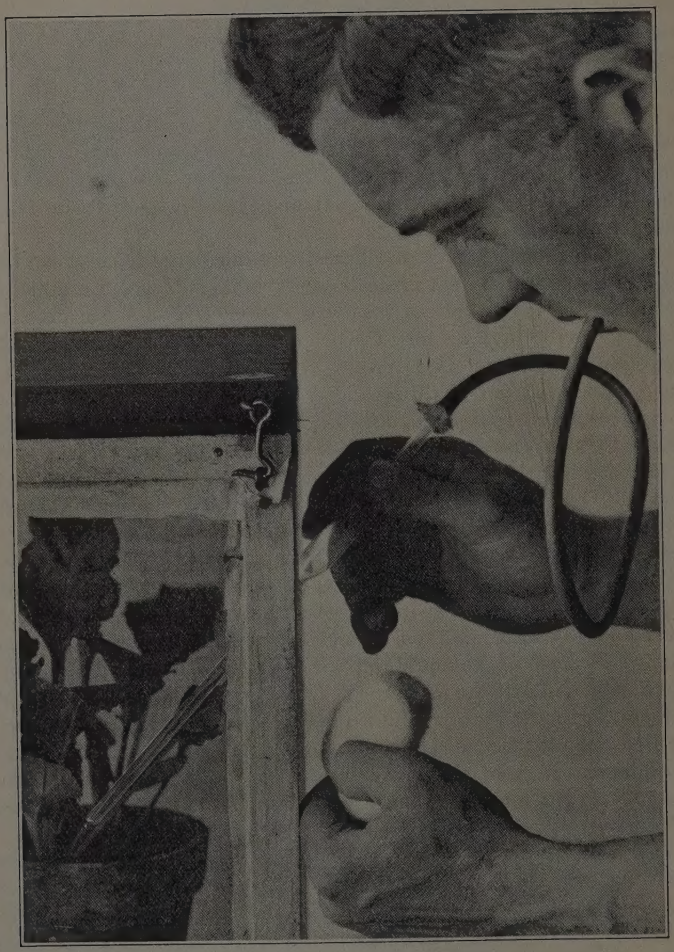

Fig. 1. Method of removing beet leafhoppers from a cage with a 10-cc pipette. By inhaling a breath of air through the rubber tube, the operator may draw the leafhoppers into the bulb of the pipette, and by exhaling he may expel them from the pipette into a vial. A piece of silk bolting covers the opening between the pipette and the rubber tubing.

Filtration.—In the filtration experiments the diseased beet juice or feeding solution containing the virus extract from the infective beet leafhoppers was centrifuged and then filtered through a coarse Berkefeld (V) or Mandler preliminary candle and refiltered through a fine Berkefeld (W), fine Mandler, or Chamberland filter. It was usually impossible to transmit curly top whenever bacterial growth developed in the filtrate owing to faulty filters or accidental aerobic contamination, and hence such preparations were not used.

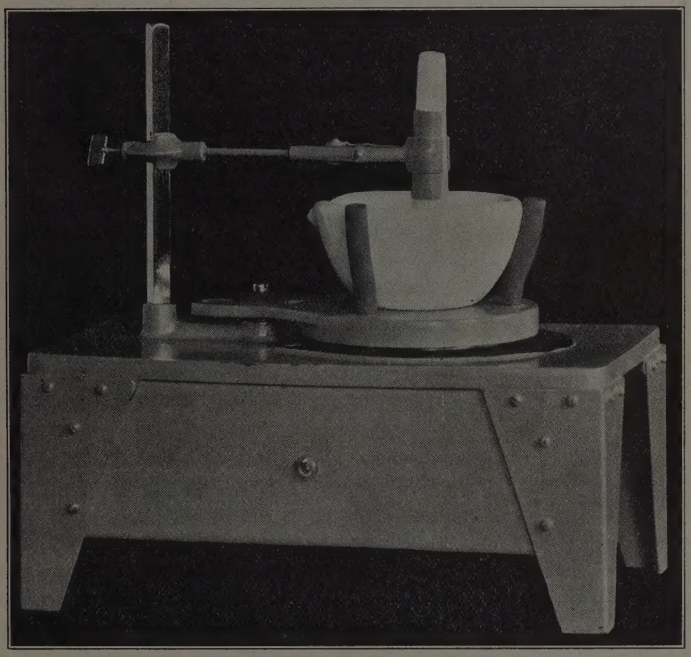

Fig. 2. Schultz electric mechanical grinder, showing mortar used in grinding beet leaves and beet leafhoppers with pulverized pyrex glass.

Feeding Beet Leafhoppers on Virus Extracts.—The methods by which the beet leafhopper can be induced to acquire the virus from virus extracts have been previously described by Carter,[9, 10, 11] Severin and Swezy,[55] and Severin.[53] The equipment for feeding noninfective nymphs consisted of a small petri or Esmarch dish (50 by 10 mm) containing about 17 cubic centimeters of the virus extract. In some experiments a stender dish (50 by 25 mm) containing about 50 cubic centimeters of the solution was used. In our early work the dish was covered

with fishskin but in later experiments Baudruche[4] transparent capping skins marked 1–A and 1–B were used. The dish was placed directly in front of the glass in a small cylindrical cage (4¾ by 5⅜ inches) covered with black sateen (fig. 3).

Feeding Period.—The noninfective nymphs were usually fasted for about 2 hours and then fed for a period of about 6 hours on unfiltered virus extracts. In the filtration experiments the insects were kept without food in empty cages during the morning, fed during the afternoon and night, and removed during the next morning, a feeding period of about 18 hours. Except in infectivity tests with single insects compared

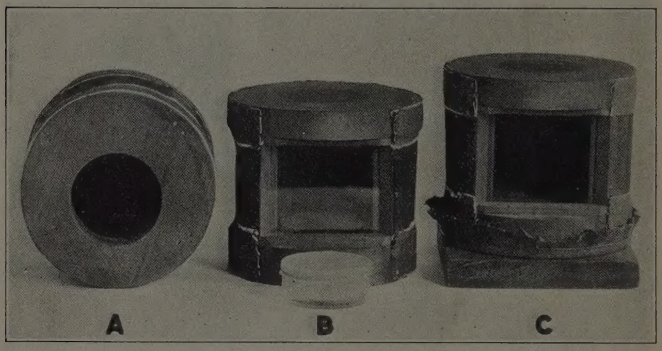

Fig. 3. Cages covered with black sateen used in feeding nymphs on virus extracts: *A*, bottom of cage showing hole off center toward the glass; *B*, stender dish covered with transparent capping skin within the cage, and also stender dish outside of cage showing height equal to that of the circular bottom board of cage; *C*, bottom of cage covered with denim and resting on a square board. The stender dish is placed directly in front of the glass in the cage and the nymphs attracted to the light come to rest on the membrane and feed.

with larger numbers, the number of nymphs used in each cage was about 100, no accurate counts being made of the number of insects. Sometimes several feeding experiments were made with each preparation. At the end of the feeding period each lot of nymphs was divided among 3 healthy beet seedlings enclosed in cages, where they remained for a period of 5 days. At the end of this time the insects were removed and the beets placed in insect-proof cages, where they were kept for a period of 3 months, if curly-top symptoms did not develop within the usual period of 10 days to 2 weeks.

Use of Noninfective Beet Leafhoppers Instead of Mechanical Inoculation.—Curly top is not readily transmitted by mechanical inoculation

[4] Baudruche capping skins are manufactured by Paul Troeder, Belleville, New Jersey.

and hence it was decided to test infectivity by feeding previously non-infective beet leafhoppers on the virus extract from diseased beets and infective leafhoppers. Severin[49] demonstrated that 6 of 72 beets and 3 of 28 beets inoculated in the crown with juice extracted from diseased leaves and beet roots respectively, developed curly top. Carsner and Stahl[8] obtained a few cases of curly top by inoculating a considerable number of healthy beets.

The results of inoculating healthy beet seedlings with curly top by means of previously noninfective leafhoppers fed on filtered and unfiltered juice from diseased beet roots have been published by Severin and Swezy.[55] They found when feeding previously non-infective nymphs at daily intervals on the filtered juice from curly-top beet roots that on the first day 67.8 to 76.1 per cent of the beets to which the insects were transferred became infected; on the second day the percentage of infection was 26.6 to 40.0, and on the third day 7.6 to 10.0 per cent. Similar tests with centrifuged, unfiltered diseased beet-root juice gave infections as follows: first day 52.9 per cent; second day 33.3 per cent; and third day none. Tests with the sediment after centrifugation gave 50 per cent infections the first day and none thereafter.

INOCULATION EXPERIMENTS WITH DISEASED BEET JUICE

Extracts from Leaves.—In the first experiment previously noninfective nymphs were fed on the extracted juice from the blades, petioles, and blades and petioles combined. The beet roots were too small to extract the quantity of juice required for the feeding equipment. The juice was expressed from the inner or youngest leaves showing symptoms of the disease from many small beets experimentally infected with curly top in the greenhouse. The leaves were ground in a food chopper and the juice was strained through several layers of cheesecloth. Some of the leaf juice was centrifuged for an hour and filtered through coarse and fine candles. The candles were changed frequently in filtering the juice since the pores became clogged, apparently by the chloroplasts.

From unfiltered juice of the blades, 30 beets were inoculated; from unfiltered petiole juice, 58 plants were inoculated; and from unfiltered juice of the leaves and petioles combined, 12 plants were inoculated. From filtrate of the blades, 9 beets were inoculated; from filtrate of the petioles, 90 beets were inoculated; and from filtrate of petioles and blades combined, 15 beets were inoculated. No infections resulted in any of these tests. Under natural conditions the beet leafhoppers obtain the

curly-top virus by feeding on the blades and petioles of small diseased beets such as those used for the extracts. A high mortality of the nymphs occurred during prolonged periods of feeding on the juices expressed from the leaves.

TABLE 1

Inoculations of Healthy Beet Seedlings with Curly Top by Means of Nymphs Fed on Diluted Juice Extracted from Leaves of Small Curly-Top Beets

Preparation No.	Diluted with equal parts of autoclaved beet-root juice and 5 per cent sugar solution		Diluted with 5 per cent sugar solution	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected
Juice from blades				
1	12	1	12	3
2	6	0	6	0
3	6	0	6	0
Total	24	1	24	3
Percentage	....	*4.2*	....	*12.5*
Juice from petioles				
4	9	2	6	0
5	6	0	9	0
6	6	3	6	1
Total	21	5	21	1
Percentage	....	*23.8*	....	*4.8*
Juice from blades and petioles combined				
7	3	0	3	1
8	9	3	9	0
9	12	8	12	3
Total	24	11	24	4
Percentage	....	*45.8*	....	*16.7*

Results summarized according to number of preparations tested and found infectious

Source of preparation	Diluted with equal parts of autoclaved beet-root juice and 5 per cent sugar solution		Diluted with 5 per cent sugar solution	
	Tested	Infectious	Tested	Infectious
Blades	3	1	3	1
Petioles	3	2	3	1
Blades and petioles combined	3	2	3	2

In the second experiment diseased blades, petioles, or blades and petioles combined were submerged before grinding in a solution in a mortar containing either 50 cc of autoclaved beet-root juice and 50 cc of 5 per cent beet sugar dissolved in sterile distilled water or 100 cc of a 5 per cent beet-sugar solution. The leaves were sometimes ground with fine sand in a mortar or with pulverized pyrex glass in a Schultz mechanical grinder. Beet sugar was added to the feeding solution because it is a favorable food for the nymphs and reduces the mortality. For inoculum

TABLE 2

INOCULATIONS OF HEALTHY BEET SEEDLINGS BY MEANS OF NYMPHS FED ON BEET-LEAF AND ROOT JUICE FROM EACH OF FIVE LARGE DISEASED BEETS

Diseased beet No.	Juice from blades		Juice from petioles		Juice from blades and petioles combined		Juice from beet roots	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
1	3	2	3	0	3	0	3	2
2	3	0	3	0	3	0	3	2
3	3	0	3	0	3	0	3	3
4	3	0	3	0	3	0	3	0
5	3	0	3	0	3	0	3	0
Total	15	2	15	0	15	0	15	7
Percentage	....	*13.3*	....	*0.0*	....	*0 0*	....	*46.7*

Results summarized according to number of preparations tested and found infectious

Juice from blades		Juice from petioles		Juice from blades and petioles combined		Juice from beet roots	
Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious
5	1	5	0	5	0	5	3

in each test the inner or youngest leaves showing symptoms of the disease were removed from 30 small beets experimentally infected with curly top in the greenhouse. The results are shown in table 1.

According to table 1, the percentage of infections was higher with diluted juice expressed from the blades and petioles combined than with the extracts from the blades or from the petioles. The summary in table 1 shows the number of extractions found to be infectious. The results seem to indicate that oxidation was a factor in the inactivation of the virus in the previous experiment, since in this experiment the leaves were submerged in the feeding solution in the process of extracting the juice.

TABLE 3

INOCULATIONS OF HEALTHY BEET SEEDLINGS WITH CURLY TOP BY MEANS OF NYMPHS FED ON CENTRIFUGED DISEASED BEET-LEAF AND ROOT JUICE

Preparation No.	Juice from blades		Juice from petioles		Juice from blades and petioles combined		Juice from beet roots	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
Centrifuged 3,500 r.p.m.								
1	6	1	6	0	15	1	9	3
2	12	7	12	0	9	0	9	7
3	6	0	6	1	9	0	6	5
4	6	0	6	0	6	0	6	0
5	6	0	6	0	6	2	6	6
6	6	0	6	0	6	0	9	8
Total	42	8	42	1	51	3	45	29
Percentage	....	*19.0*	....	*2.4*	....	*5.9*	....	*64.4*
Supercentrifuged 40,000 r.p.m.								
1	12	3	12	3	12	0	12	10
2	9	0	9	0	9	0	9	5
3	9	0	9	0	9	0	9	2
4	9	1	9	0	9	0	9	3
5	9	0	9	0	9	0	9	1
6	9	0	9	0	9	0	9	3
Total	57	4	57	3	57	0	57	24
Percentage	....	*7.0*	....	*5.3*	....	*0.0*	....	*42.1*
Supercentrifuged 40,000 r.p.m.*								
1	12	0	12	1	12	0	12	11
2	9	3	9	0	9	0	27	10
Total	21	3	21	1	21	0	39	21
Percentage	....	*14.3*	....	*4.8*	....	*0.0*	....	*53.8*

Results summarized according to number of preparations tested and found infectious

Centrifugation	Juice from blades		Juice from petioles		Juice from blades and petioles combined		Juice from beet roots	
	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious
3,500	6	2	6	1	6	2	6	5
40,000	6	2	6	1	6	0	6	6
40,000*	2	1	2	1	2	0	2	2

* Leaf and root juice which remained in supercentrifuge after centrifugation in preparations 1 and 2.

Extract from Beet Root.—In the first two experiments no tests were made with root juice since the beets were too small to yield a sufficient quantity of juice, and hence in the third experiment large diseased beets removed from the field were used. The juice was expressed from the blades, petioles, blades and petioles combined, and beet roots from each of 5 large beets in an advanced stage of the disease. The results of inoculating healthy beet seedlings by means of previously noninfective nymphs which were fed on the extracts are indicated in table 2.

According to table 2 infections were obtained from extracts of diseased blades and root of beet No. 1, from the roots of beets Nos. 2 and 3, but no infections were obtained with root juice expressed from beets 4 and 5. The summary in table 2 shows that 1 of 5 preparations from the blades and 3 of 5 preparations from the beet roots were infectious. No infections were obtained with preparations from the petioles or with blades and petioles combined.

Centrifuged Beet Juice.—In the fourth experiment the extracts from blades, petioles, blades and petioles combined, and roots of large beets removed from the field were centrifuged for 1 hour at 3,500 revolutions per minute and other portions of each extract were supercentrifuged at 40,000 revolutions per minute. Supercentrifugation of leaf juices removed most of the chloroplasts. The results of inoculating healthy beet seedlings with curly top by means of previously noninfective nymphs fed on the centrifuged and supercentrifuged beet-leaf and root juices are shown in table 3.

That the virus can be more readily transmitted with centrifuged and supercentrifuged beet-root juice than with leaf juice seems clearly indicated in table 3. The summary in table 3 shows no marked difference in the number of preparations found infectious with centrifuged and with supercentrifuged beet-root and leaf juices.

Diluted Centrifuged Beet Juice.—In the fifth experiment extracts from diseased leaves and beet roots were diluted and then centrifuged for 1 hour at 3,500 revolutions per minute. Four different diluents were used, in each case equal parts of diseased beet juice and diluents being mixed. The diluents were: (1) autoclaved filtered beet-root juice; (2) equal parts of autoclaved filtered beet-root juice and a 5 per cent solution of beet sugar; (3) a 5 per cent solution of beet sugar; and (4) sterile distilled water. The infections obtained are indicated in table 4.

In two tests indicated in table 4 the infection with diluted diseased blade juice was higher, 66.7 per cent as compared with 53.3 per cent with diluted diseased beet-root juice. All other percentages with diluted blade, petiole, and blade and petiole juice combined were lower than with the same dilution of diseased beet-root juice. The summary in table

TABLE 4

INOCULATIONS OF HEALTHY SUGAR BEET SEEDLINGS WITH CURLY TOP BY MEANS OF NYMPHS FED ON DILUTED DISEASED BEET-LEAF AND ROOT JUICE

Preparation No.	Juice from blades		Juice from petioles		Juice from blades and petioles combined		Juice from beet roots	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
Equal parts of diseased juice and autoclaved filtered beet-root juice								
1	6	4	6	0	6	0	6	1
2	6	1	6	0	6	0	6	1
3	6	4	6	1	6	2	6	6
4	6	0	6	0	6	0	6	3
Total	24	9	24	1	24	2	24	11
Percentage	....	*37.5*	....	*4.2*	....	*8.3*	....	*45.8*
Equal parts of diseased juice and a mixture of equal parts of autoclaved beet-root juice and a 5 per cent sugar solution								
1	3	3	3	0	3	0	6	4
2	3	1	3	0	3	0	9	4
Total	6	4	6	0	6	0	15	8
Percentage	....	*66.7*	....	*0.0*	....	*0.0*	....	*53.3*
Equal parts of diseased juice and a 5 per cent sugar solution								
1	3	1	3	0	3	0	9	7
2	3	0	3	0	3	0	6	6
Total	6	1	6	0	6	0	15	13
Percentage	....	*16.7*	....	*0.0*	....	*0.0*	....	*86.7*
Equal parts of diseased juice and distilled water								
1	6	0	6	1	6	0	6	1
2	6	0	6	2	6	0	6	2
3	6	0	6	0	6	0	6	1
4	6	0	6	0	6	0	6	4
Total	24	0	24	3	24	0	24	8
Percentage	....	*0.0*	....	*12.5*	....	*0.0*	....	*33.3*

Results summarized according to number of preparations tested and found infectious

Diluent	Juice from blades		Juice from petioles		Juice from blades and petioles combined		Juice from beet roots	
	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious
Autoclaved filtered beet-root juice	4	3	4	1	4	1	4	4
Autoclaved beet-root juice and a sugar solution	2	2	2	0	2	0	2	2
Sugar solution	2	1	2	0	2	0	2	2
Distilled water	4	0	4	2	4	0	4	4

4 shows that 6 of 12 preparations of blade juice, 3 of 12 preparations of petiole juice, 1 of 12 preparations of blade and petiole juice combined, and all 12 preparations from beet roots were infectious. The dilution tests, however, do not reveal an explanation for the difference in results obtained with the extracts from the leaves and beet root.

AGING OF VIRUS IN BEET-ROOT JUICE

In Extracted, Centrifuged, and Supercentrifuged Preparations.—Tests were made to determine whether the curly-top virus in diseased beet-root juice was inactivated more rapidly by exposure to the air at room temperature in a small petri or Esmarch dish (50 by 10 mm) containing 17 cc or in a stender dish (50 by 25 mm) containing 50 cc. In each test the extract from large diseased beet roots was divided into 3 portions; one part was not centrifuged; another portion was centrifuged for a period of 1 hour at 3,500 revolutions per minute; and the last part was supercentrifuged at 40,000 revolutions per minute, using the coarse nozzle once and the fine nozzle twice. The results obtained are shown in table 5.

An inactivation of the virus occurred after the beet-root juice was exposed to the air at room temperatures for a period of 72 hours, as is shown in table 5. In some of the tests, except with supercentrifuged beet-root juice, the virus extract became thick and jelly-like owing to bacterial growth, within 16 to 76 hours, according to temperature. The lowest percentage of infection was obtained with extracted beet-root juice in an Esmarch dish containing 17 cubic centimeters.

In Aerobic and Anaerobic Filtrates.—The longevity of the curly-top virus was determined by aging the filtrate prepared from the juice of diseased beet roots under aerobic and anaerobic conditions. The aerobic filtrate was kept in sterile test tubes plugged with cotton, while in other test tubes the surface of the filtrate was capped with a mixture of equal parts of hot melted paraffin and crude vaseline to prevent oxidation and provide partially anaerobic conditions. Previously noninfective nymphs were fed at daily intervals on the aerobic and partially anaerobic filtrates and then the insects were transferred to 3 healthy beet seedlings in each test. Table 6 shows the results obtained.

According to table 6 the transmission of curly top varied as follows: aerobic filtrate 13.3 to 66.7 per cent; anaerobic filtrate 19.0 to 70.8 per cent. The highest percentages of infections were obtained during the first 2 days with the aerobic filtrate and during the first 3 days with the anaerobic filtrate.

Table 6 shows that the virus was recovered from 4 of 5 aerobic preparations on each of the first, second, and third days; from 3 of 5 on the fourth and fifth days; from none on any of the succeeding days except from 1 of 5 on the eighth day. With the anaerobic filtrate the virus was reclaimed from 5 of 5 preparations tested on the first, second, and third

TABLE 5

EFFECT OF EXPOSING VIRUS EXTRACT FROM DISEASED BEET ROOTS TO THE AIR AT ROOM TEMPERATURE

Number of hours exposed to air	Extracted beet-root juice				Centrifuged beet-root juice 3,500 r.p.m. 1 hour				Supercentrifuged beet-root juice 40,000 r.p.m.			
	17 cc		50 cc		17 cc		50 cc		17 cc		50 cc	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
2	....	....	3	2	....	....	3	2	....	....	....	....
3	....	....	3	1	....	....	3	2	....	....	....	..
4	....	....	6	3	....	....	6	3	....	....	3	0
5	....	....	3	0	....	..	3	0	....	....	....	..
6	....	....	6	3	....	....	6	4	....	....	3	3
7	....	....	3	0	....	..	3	1	....	....	....	....
8	....	....	6	2	....	....	6	4	....	....	3	0
9	....	....	3	1	....	....	3	0	....	....	....	....
10	3	0	6	0	....	....	3	0	....	....	3	0
12	3	0	3	3	....	....	3	1	....	....	3	1
14	3	0	3	1	....	....	3	1	....	....	3	1
16	6	0	6	1	3	0	6	0	3	1	6	4
17	6	1	6	3	6	3	6	3	6	4	6	6
18	3	0	3	0	3	0	6	0	3	0	3	0
19	6	3	6	1	6	4	6	3	6	1	6	1
20	3	0	3	1	3	0	3	0	3	1	3	0
21	6	3	6	3	6	3	6	3	6	3	6	3
22	3	0	3	0	3	0	3	0	3	1	3	0
23	6	1	6	3	6	4	6	2	6	4	6	6
24	3	0	3	0	3	0	3	0	3	0	3	1
25	6	3	6	1	6	3	6	1	6	2	6	2
26	6	4	6	4	6	4	6	3	6	2	6	0
28	6	3	6	3	6	2	6	4	6	2	6	3
30	6	0	6	3	6	3	6	0	6	3	6	3
32	6	1	6	4	6	1	6	1	6	3	6	1
40	6	0	6	0	6	0	6	0	6	0	6	1
42	6	0	6	0	6	0	6	0	6	0	6	0
44	6	0	6	1	6	0	6	0	6	0	6	1
46	6	0	6	0	6	0	6	0	6	0	6	0
48	12	3	12	1	12	3	12	2	12	3	12	0
50	6	0	6	1	6	0	6	0	6	1	6	1
52	6	0	6	0	6	0	6	0	6	0	6	0
54	3	0	3	0	3	0	3	1	3	0	3	0
72	6	0	6	0	6	0	6	0	6	0	6	0
74	6	0	6	0	6	0	6	0	6	0	6	0
76	6	0	6	0	6	0	6	0	6	0	6	0
Total	150	22	186	46	138	30	186	41	138	31	159	38
Percentage	....	*14.7*	....	*24.7*	....	*21.7*	....	*22.0*	....	*22.5*	....	*23.9*

day; from 4 of 5 on the fourth day; 2 of 5 on the fifth day; 4 of 5 on the sixth day; 5 of 5 on the seventh day; 3 of 4 on the eighth day; and 1 of 2 on the ninth day.

TABLE 6

DAILY INOCULATIONS OF HEALTHY BEET SEEDLINGS WITH CURLY TOP BY MEANS OF NYMPHS FED ON AEROBIC AND PARTIALLY ANAEROBIC FILTRATES PREPARED FROM JUICE EXTRACTED FROM DISEASED BEET ROOTS

Age of preparation, days	Preparation No. 1		Preparation No. 2		Preparation No. 3		Preparation No. 4		Preparation No. 5		Totals		Per cent	Number of preparations	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected		Tested	Infectious
Aerobic filtrate															
1	3	2	3	0	3	3	3	2	3	2	15	9	*60.0*	5	4
2	3	0	3	2	3	3	3	3	3	2	15	10	*66.7*	5	4
3	3	2	3	0	3	3	3	1	3	1	15	7	*46.7*	5	4
4	3	2	3	0	3	3	3	0	3	3	15	8	*53.3*	5	3
5	3	1	3	0	3	2	3	0	3	2	15	5	*33.3*	5	3
6	3	0	3	0	3	0	3	0	3	0	15	0	*0.0*	5	0
7	3	0	3	0	3	0	3	0	3	0	15	0	*0.0*	5	0
8	3	0	3	0	3	0	3	2	3	0	15	2	*13.3*	5	1
9	3	0	3	0	3	0	3	0	3	0	15	0	*0.0*	5	0
10	3	0	3	0	3	0	3	0	3	0	15	0	*0.0*	5	0
Anaerobic filtrate															
1	6	5	6	3	6	4	3	3	3	2	24	17	*70.8*	5	5
2	6	2	6	1	6	5	3	2	3	3	22	13	*59.1*	5	5
3	6	2	6	1	3	3	3	3	3	3	21	12	*57.1*	5	5
4	3	1	6	2	6	3	3	3	3	0	21	9	*42.9*	5	4
5	3	0	6	0	6	2	3	2	3	0	21	4	*19.0*	5	2
6	3	2	6	2	6	3	3	0	3	2	21	9	*42.9*	5	4
7	3	1	6	2	6	2	3	2	3	3	21	10	*47.6*	5	5
8	3	2	6	0	....	....	3	3	3	2	15	7	*46.7*	4	3
9	3	3	3	0	....	....	....	....	....	....	6	3	*50.0*	2	1
10	....	....	....	....	....	....	....	....	....	....	....	....	.....	....	....

In later experiments weekly inoculations were made with partially anaerobic filtrates prepared from supercentrifuged diseased beet-root juice, and infections were obtained at the end of 5 weeks as shown in table 7.

In Filtrate Prepared from Diluted Supercentrifuged Preparations.—An experiment was conducted with partially anaerobic filtrates prepared from supercentrifuged diseased beet-root juice. In one test the beet-root juice was diluted with equal parts of sterile distilled water and in another test the extract was not diluted. Each was supercentrifuged

at 40,000 revolutions per minute. The filtrate in test tubes was capped with equal parts of paraffin and vaseline. Table 7 shows the results of weekly inoculations during a period of 10 weeks.

TABLE 7

WEEKLY INOCULATIONS OF HEALTHY BEET SEEDLINGS WITH CURLY TOP BY MEANS OF NYMPHS FED ON PARTIALLY ANAEROBIC FILTRATES PREPARED FROM DILUTED AND UNDILUTED SUPERCENTRIFUGED DISEASED BEET-ROOT JUICE

Age of filtrate, weeks	Filtrate prepared from undiluted juice		Filtrate prepared from juice diluted 1:1 with distilled water	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected
1	6	6	6	2
2	3	2	3	2
3	3	0	3	3
4	3	1	3	0
5	3	1	3	0
6	3	0	3	0
7	3	0	3	0
8	3	0	3	0
9	3	0	3	0
10	3	0	3	0

According to table 7 infections were obtained with the diluted partially anaerobic filtrate at the end of 3 weeks and with the partially anaerobic filtrate at the end of 5 weeks. The age of the virus was not increased in the partially anaerobic filtrate prepared from the diluted supercentrifuged diseased beet-root juice as compared with the partially anaerobic filtrate prepared from the undiluted supercentrifuged root juice.

Effect of pH on Aging Under Anaerobic Conditions.—The effect of aging on the curly-top virus was determined with the filtrate prepared from diseased beet-root juice placed in anaerobic jars. The extract was adjusted to a pH range from 6.4 to 2.9 and then filtered through coarse and refiltered through fine Berkefeld candles. Anaerobic conditions were produced by exhausting the air in the jars with hydrogen, and by the use of pyrogallolic acid placed on the bottom of the jars. It is questionable whether strictly anaerobic conditions prevailed in the jars. The filtrates used as a control were fed to previously noninfective nymphs a few hours after the pH was adjusted. Table 8 shows the results obtained during a period of 100 days.

In beet-root juice adjusted to pH 3.5 the virus was apparently inactivated the first time it was tested at the end of 7 days, while with the same juice at pH 5.0 and pH 6.0 the virus was active after 100 days.

TABLE 8

EFFECT OF pH ON AGING UNDER PARTIALLY ANAEROBIC CONDITIONS

Age of filtrate, days	Preparation No.	pH 6.0*		pH 5.0		pH 4.0		pH 3.5		pH 3.0†	
		Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
0	1	3	3	3	3	3	3	....	....	3	0
	2	3	1	3	3	3	3	....	....	3	0
	3	3	1	3	3	3	1	....	....	3	0
3	1	6	2	6	5	6	0	....	....	6	0
	2	3	0	3	1	3	0	....	....	3	0
4	3	3	2	6	0	6	1	....	....	6	0
7	1	6	0	6	5	6	0	....	....	6	0
	2	6	6	6	6	....	....	....	....	....	....
	3	3	1	3	0	3	0	....	....	3	0
	4	3	1	3	2	....	....	3	0	....	....
10	2	3	0	6	3	6	0	....	....	....	....
14	4	3	3	3	3	....	....	3	0	....	....
22	4	3	2	3	2	....	....	3	0	....	....
34	4	6	5	3	2	....	....	6	0	....	....
71	4	3	2	3	3	....	....	3	0	....	....
100	4	6	3	6	4	....	....	3	0	....	....

* Preparation No. 1 had a pH of 6.4, No. 2 a pH of 6.3, and Nos. 3 and 4 a pH of 6.0.

† Preparation Nos. 1 and 2 had a pH of 2.9, No. 3 a pH of 3.0.

CULTIVATION OF VIRUS OUTSIDE OF LIVING PLANT

Olitisky[43, 44] came to the conclusion that the virus of tobacco and tomato mosaic is a living, multiplying, microbic body which can be cultivated and is capable of propagating itself through many generations in an artificial medium. He obtained infections with the twelfth subculture representing a dilution magnitude of 4×10^{-16} whereas the dilution limit was 10^{-6}.

Mulvania,[41] Purdy,[46] Goldsworthy,[21] Smith,[56] and Grainger[23] have tried to repeat this experiment but without success.

Tests were made to determine whether the curly-top virus could be cultivated in a feeding solution under anaerobic conditions. The feeding solution consisted of 300 cc of sterile beet-root juice to which was added 50 cc of a 2 per cent solution of beet sugar and 50 cc of a 2 per cent solution of soluble starch. After the test tubes containing the filtered diseased beet-root juice adjusted to pH 6.0 and pH 5.0 were removed from the anaerobic jar at the end of 100 days in the previous experiments, 1 loop or 1 cc was transplanted in 16 cc of the feeding solution. The first

transplants were incubated in an anaerobic jar for a period of 10 days and the second transplants for a period of 10 additional days. Noninfective nymphs after feeding on the first and second transplants failed to transmit curly top to healthy beet seedlings as indicated in table 9. Aging the virus under anaerobic conditions probably reduces its virulence.

TABLE 9

ATTEMPT TO CULTIVATE CURLY-TOP VIRUS IN A FEEDING SOLUTION UNDER ANAEROBIC CONDITIONS

Preparation No.	Transplant	Quantity of filtrate transplanted	Period of incubation, days	pH 6		pH 5	
				Beets inoculated	Beets infected	Beets inoculated	Beets infected
1	First	1 loop	10	3	0	3	0
	Second	1 loop	20	3	0	3	0
2	First	1 cc	10	3	0	3	0
	Second	1 cc	20	3	0	3	0
3	First	1 cc	10	3	0	3	0
	Second	1 cc	20	3	0	3	0

RESISTANCE OF VIRUS TO DRYING IN PLANT TISSUES AND IN INFECTIVE BEET LEAFHOPPERS

The curly-top virus was inactivated in dried diseased beet pulp and beet roots. In one experiment 40 diseased beet roots were ground in a meat grinder and the pulp was slowly dried in the greenhouse for a period of 5 weeks and in the headhouse where the light was less intense for a period of 7 weeks. In another experiment the beet roots of 20 plants, in an advanced stage of the disease, were dried in the greenhouse for a period of 7 weeks. In both experiments the dried plant tissue was steeped in equal parts of steam-extracted beet-root juice and sterile distilled water containing 5 per cent beet sugar. Noninfective nymphs after feeding on the filtered and unfiltered beet-root extracts failed to transmit curly top to healthy beet seedlings.

Dried infective beet leafhoppers were pulverized in a mortar and steeped in a feeding solution similar to that used in the preceding experiment. Noninfective nymphs after feeding on the filtered and unfiltered extract, were transferred to healthy beet seedlings, but no curly top developed.

INACTIVATION OF VIRUS WITH JUICES FROM IMMUNE HOST PLANT

It has been found that it is not possible to transmit curly top to any plant of the Gramineae, or grass family. Alameda or Mammoth sweet corn *(Zea mays)* is a favorable food plant of the beet leafhopper but is unfavorable to the curly-top virus. It was decided to express the juice from healthy sweet corn plants and determine whether the curly-top virus in beet-root juice was inactivated in the extract from sweet-corn plants. The sweet-corn and beet-root juices were centrifuged for 1 hour at 3,500 revolutions per minute except in one test indicated in table 10, in which corn juice was supercentrifuged at 40,000 revolutions per minute. Various dilutions were used and a period of 2, 4, or 6 hours elapsed before exposing the previously noninfective nymphs to the feeding solution. In two tests the nymphs were exposed to the feeding solution immediately after the dilutions were made. The controls were diluted with sterile distilled water. The results are indicated in table 10.

It is evident from table 10 that infections were obtained with diseased beet-root juice diluted with sweet-corn juice as follows: 4:1, 2:1, 1:1, and 1:2. No infections were obtained with dilutions of 1:50, 1:100, and 1:200 when a period of 2, 4, and 6 hours elapsed before exposing the previously noninfective nymphs to the feeding solution.

Starrett[58] found in her studies on the transmission of curly top to *Oxalis stricta* that the acidity for normal leaves and young stems of the species is pH 2.45, and for the young leaves it is pH 2.23. The juice extracted from corn plants was pH 5.5. In all probability there are other factors in corn juice which inactivate the curly-top virus.

PURIFICATION OF VIRUS

McKinney[40] and Brewer, Kraybill, Samson, and Gardner[6] have attempted purification methods with mosaic diseases. The best success was obtained with the residue from supercentrifuged juice.

Similar supercentrifuging tests were made with extracts from the blades and petioles combined; blades, petioles, and beet root combined; and beet root. The leaves and beet roots were ground in a food chopper and the juices were expressed through muslin. The juice was diluted with an equal volume of distilled water and passed through a supercentrifuge at a speed of 40,000 revolutions per minute. The diluted juice was supercentrifuged three times, using the coarse nozzle the first time and the fine nozzle the second and third times. The gummy residue was

scraped from the bowl of the supercentrifuge, resuspended in distilled water equal to the original volume of the beet juice, and passed through the supercentrifuge using the fine nozzle. The second residue formed in

TABLE 10

Inoculations of Healthy Beet Seedlings with Curly Top by Means of Nymphs Fed on Diseased Beet-Root Juice Diluted with Juice Expressed from Sweet-Corn Plants

Experiments with lower dilutions

Period elapsed before feeding nymphs, hours	Preparation No.	Diluent	Ratio of diseased beet-root juice to diluent									
			4:1		2:1		1:1		1:2		1:4	
			Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
0	1	Water	3	2	3	2	3	3	....	....	....	....
		Corn juice	3	3	3	3	3	2	....	....	....	....
	2	Corn juice	3	0	3	0	3	0	....	....	....	....
	3	Corn juice	3	3	3	2	3	0	....	....	....	....
	4	Water	....	....	....	....	3	1	3	2	3	2
		Corn juice	....	....	....	....	3	0	3	0	3	1
2	4	Water	....	....	....	....	3	2	3	0	3	3
		Corn juice	....	....	....	....	3	0	3	0	3	1
4	4	Water	....	....	....	....	3	2	3	1	3	1
		Corn juice	....	....	....	....	3	0	3	1	3	0

Experiments with higher dilutions

Period elapsed before feeding nymphs, hours	Preparation No.	Diluent	Ratio of diseased beet-root juice to diluent							
			1:1		1:50		1:100		1:200	
			Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
2	1	Water	3	2	3	0	3	1	3	0
		Corn juice*	3	1	3	0	3	0	3	0
4	2	Water	3	1	3	1	3	2	3	0
		Corn juice*	3	0	3	0	3	0	3	0
2	3	Water	....	....	3	3	3	1	3	1
		Corn juice	....	....	3	0	3	0	3	0
4	4	Water	....	....	3	1	3	1	3	0
		Corn juice	....	....	3	0	3	0	3	0
6	5	Water	....	....	3	1	3	1	3	0
		Corn juice	....	....	3	0	3	0	3	0

* Corn juice supercentrifuged 40,000 r.p.m.

the bowl was discarded. Aluminum gel adjusted to pH 5.8 and 6.2 was added to the supercentrifuged liquid and the mixture was filtered through coarse and fine candles. The results of feeding previously non-infective nymphs on the liquid after each supercentrifugation and on the filtrate are shown in tables 11 and 12.

TABLE 11

INOCULATIONS OF HEALTHY BEET SEEDLINGS WITH CURLY TOP BY MEANS OF NYMPHS FED ON DILUTED EXTRACTS FROM DISEASED BEETS SUPERCENTRIFUGED THREE TIMES AND FOURTH TIME WITH GUMMY RESIDUE RESUSPENDED IN DISTILLED WATER

Source of preparation	Preparation No.	Number of supercentrifugations							
		1		2		3		4	
		Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
Blades and petioles	1	3	0	3	1	3	0	3	0
	2	3	1	3	0	3	0	3	1
		—	—	—	—	—	—	—	—
	Total	6	1	6	1	6	0	6	1
	Percentage	....	*16.7*	....	*16.7*	....	*0.0*	....	*16.7*
Blades, petioles, and beet roots	3	3	0	3	1	3	1	3	1
	4	3	1	3	0	3	0	3	0
	5	3	1	3	2	3	1	3	0
		—	—	—	—	—	—	—	—
	Total	9	2	9	3	9	2	9	1
	Percentage	....	*22.2*	....	*33.3*	....	*22.2*	....	*11.1*
Beet roots	6	3	1	3	1	3	1	3	1
	7	3	0	3	1	3	3	3	1
	8	3	0	3	1	3	0	3	1
	9	3	1	3	1	3	2	3	2
	10	3	1	3	0	3	1	3	0
		—	—	—	—	—	—	—	—
	Total	15	3	15	4	15	7	15	5
	Percentage	....	*20.0*	....	*26.7*	....	*46.7*	....	*33.3*

Results summarized according to number of preparations tested and found infectious

Source of preparation	Number of supercentrifugations							
	1		2		3		4	
	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious
Blades and petioles	2	1	2	1	2	0	2	1
Blades, petioles, and beet roots	3	2	3	2	3	2	3	1
Beet roots	5	3	5	4	5	4	5	4

According to Brewer *et al.*[6] the juice from typical tomato mosaic plants after centrifugation three times should contain relatively little virus. The gummy residue resuspended in distilled water, according to McKinney's[40] supercentrifugation tests with tobacco mosaic, contained most of the virus.

TABLE 12

Inoculations of Healthy Beet Seedlings with Nymphs Fed on Filtrate Containing a Mixture of Supercentrifuged Liquid Prepared from Gummy Residue of Diseased Beet-Root Juice and Aluminum Gel

Age of filtrate	Amount of aluminum gel in 100 cc of filtrate					
	1 cc		5 cc		10 cc	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
1 hour	....	....	6	1	6	0
1½ hours	....	....	3	0	3	0
2 hours	3	1	6	1	6	2
1 day	3	1	3	0	3	0
5 days	....	....	3	0	3	0
8 days	....	....	3	0	3	0
2 weeks	....	....	3	0	3	0
3 weeks	....	....	3	0	3	0
4 weeks	....	....	3	0	3	0
5 weeks	....	....	3	0	3	0
6 weeks	....	....	3	0	3	0

It is evident from table 11 that infections were obtained with beet extracts, with one exception, after each supercentrifugation. There was no evidence to show that an increase in the number of infections occurred with the supercentrifuged liquid obtained from the gummy residue resuspended in distilled water. No infections were obtained after the first day with the filtrate containing a mixture of supercentrifuged liquid prepared from the gummy residue and aluminum gel, as indicated in table 12.

DILUTION OF VIRUS FROM DISEASED BEET ROOTS

Three experiments were conducted in determining the tolerance to dilution of the curly-top virus in diseased beet-root juice using extracted, centrifuged, and filtered juice. Conical beakers or ordinary beakers were used in this work, and the dilutions were made with pipettes. The diluent consisted of sterile distilled water. The diluted juice was thoroughly agitated by a circular movement of the beaker and by pouring the solution back and forth in 2 beakers. A stender dish (50 by 25 mm) containing about 50 cc of diluted juice was used in the feed-

ing experiments. An undiluted control was used in each experiment. Tables 13–15 show the results obtained in the three experiments.

In Extracted Juice.—The tolerance to dilution of extracted beet-root juice was 1:100 as indicated in table 13. The percentage of infections was lower with the undiluted control than with dilutions of 1:10, 1:25,

TABLE 13

INOCULATION OF HEALTHY BEET SEEDLINGS WITH CURLY TOP BY MEANS OF NYMPHS FED ON DILUTED EXTRACTED DISEASED BEET-ROOT JUICE

Preparation No.	Undiluted control		Dilutions of extracted diseased beet-root juice											
			1:10		1:25		1:50		1:100		1:200		1:300	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
1	3	0	3	1	3	1	3	1	3	0	....	....	....	....
2	3	0	3	0	3	0	3	0	3	0	..	....	....	....
3	3	0	6	1	6	4	6	1	3	0	...	...	....	....
4	3	1	....	....	....	....	....	....	3	0	3	0	3	0
5	3	1	....	....	....	....	....	....	3	0	6	0	12	0
6	3	0	....	....	....	....	....	....	9	1	9	0	9	0
Total	18	2	12	2	12	5	12	2	24	1	18	0	24	0
Percentage	....	*11.1*	....	*16.7*	....	*41.7*	....	*16.7*	....	*4.2*	....	*0.0*	....	*0.0*

Results summarized according to number of preparations tested and found infectious

	Undiluted control		Dilutions of extracted diseased beet-root juice											
			1:10		1:25		1:50		1:100		1:200		1:300	
	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious
	6	2	3	2	3	2	3	2	6	1	3	0	3	0

and 1:50. A high mortality of the nymphs often occurred when fed on undiluted extracted beet-root juice used as a control. When previously noninfective nymphs failed to obtain the infective dose from a preparation in both the undiluted control and dilutions, the test of that preparation was not included in table 13; there were three such preparations with the extracted juice. On the other hand, when infections were obtained with the undiluted control and not with the dilutions or vice versa, the tests were included in the table. The summary in table 13 shows that 2 of 6 preparations of extracted diseased beet-root juice used

TABLE 14

INOCULATION OF HEALTHY BEET SEEDLINGS WITH CURLY TOP BY MEANS OF NYMPHS FED ON DILUTED, CENTRIFUGED, DISEASED BEET-ROOT JUICE

Preparation No.	Undiluted control		Dilutions of centrifuged diseased beet-root juice											
			1:10		1:25		1:50		1:100		1:200		1:300	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
1	9	2	3	2	3	1	3	0	3	0	3	0	....	....
2	3	3	3	3	3	3	3	3	3	3	3	2	....	....
3	3	3	3	3	3	3	3	2	3	2	3	3	....	....
4	3	3	....	....	....	....	....	....	3	1	3	0	3	0
5	3	3	....	....	....	....	....	....	3	1	3	2	3	1
6	3	2	....	....	....	....	....	....	3	0	3	1	3	0
7	3	3	....	....	....	....	....	....	3	2	3	0	3	1
8	6	6	....	....	....	....	....	....	3	2	3	2	3	3
Total	33	25	9	8	9	7	9	5	24	12	24	10	15	5
Percentage	....	*75.8*	....	*88.9*	....	*77.8*	....	*55.6*	....	*50.0*	....	*41.7*	....	*33.3*

Preparation No.	Dilutions of centrifuged diseased beet-root juice													
	1:400		1:500		1:600		1:700		1:800		1:900		1:1,000	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
1	....	....	....	....	3	2	3	0	3	0	3	0	3	0
2	....	....	....	....	3	1	3	0	3	0	3	0	3	0
3	....	....	....	....	3	0	3	0	3	0	3	0	3	0
4	3	0	3	1	3	0	3	0	3	0	3	0	3	0
5	3	0	3	0	3	0	3	0	3	0	3	0	3	0
6	3	0	3	0	3	0	3	0	3	0	3	0	3	0
7	3	0	3	0	3	0	3	0	3	0	3	0	3	0
8	3	2	3	2	3	2	3	1	3	1	3	2	3	1
Total	15	2	15	3	27	5	24	1	24	1	24	2	24	1
Percentage	....	*13.3*	....	*20.0*	....	*18.5*	....	*4.2*	....	*4.2*	....	*8.5*	....	*4.2*

Results summarized according to number of preparations tested and found infectious

	Undiluted control		Dilutions of centrifuged diseased beet-root juice											
			1:10		1:25		1:50		1:100		1:200		1:300	
	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious
	8	8	3	3	3	3	3	2	8	6	8	5	5	3

	Dilutions of centrifuged diseased beet-root juice													
	1:400		1:500		1:600		1:700		1:800		1:900		1:1,000	
	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious
	5	1	5	2	8	3	8	1	8	1	8	1	8	1

TABLE 15

INOCULATION OF HEALTHY BEET SEEDLINGS WITH CURLY TOP BY MEANS OF NYMPHS FED ON DILUTED, FILTERED, DISEASED BEET-ROOT JUICE

Filter candles used	Preparation No.	Undiluted control		Dilutions of filtrate prepared from diseased beet-root juice							
				1:10		1:25		1:50		1:100	
		Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
Berkefeld V, W	1	3	2	3	1	3	2	3	3	3	2
	2	3	1	3	2	3	3	3	2	3	2
	3	3	2	3	3	3	1	3	3	3	0
	4	6	1	6	6	3	1	3	1	3	1
	5	3	0	....	....	....	....	3	0	3	0
Mandler 2 to 5, 10 to 16	6	3	3	....	....	....	....	3	0	3	1
	7	3	3	....	....	....	....	3	0	3	1
Berkefeld V, W; Chamberland L 13	8	6	2	3	0	3	1	3	0	3	0
Total	....	30	14	18	12	15	8	24	9	24	7
Percentage	....	....	*46.7*	....	*66.7*	....	*53.3*	....	*37.5*	....	*29.2*

Filter candles used	Preparation No.	Dilutions of filtrate prepared from diseased beet-root juice							
		1:200		1:300		1:400		1:500	
		Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
Berkefeld V, W	1	....	....	....	....	....	....	3	0
	2	....	....	....	....	....	....	3	0
	3	....	....	....	....	....	....	3	0
	4	3	2	3	2	3	0	3	0
	5	3	1	3	0	3	0	3	0
Mandler 2 to 5, 10 to 16	6	3	0	3	0	3	0	3	0
	7	3	0	....	....	3	0	3	0
Berkefeld V, W; Chamberland L 13	8	3	0	....	....	....	....	....	....
Total	....	15	3	9	2	12	0	21	0
Percentage	....	....	*20.0*	....	*22.2*	....	*0.0*	....	*0.0*

Results summarized according to number of preparations tested and found infectious

Undiluted control		Dilutions of filtrate prepared from diseased beet-root juice							
		1:10		1:25		1:50		1:100	
Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious
8	7	5	4	5	5	8	4	8	5

Dilutions of filtrate prepared from diseased beet-root juice

1:200		1:300		1:400		1:500	
Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious
5	2	3	1	4	0	7	0

as an undiluted control were infectious; 2 of 3 preparations in each dilution of 1:10, 1:25, and 1:50, and 1 of 6 preparations diluted 1:100, were infectious.

In Centrifuged Juice.—The tolerance to dilution with centrifuged diseased beet-root juice was 1:1,000 and was obtained with 49 small beets removed from the field during the spring. Infections were also obtained at intervals of 100, from 100 to 1,000 with centrifuged root juice extracted from the same 49 diseased beets as is shown in table 14. With undiluted centrifuged diseased beet-root juice used as a control 75.8 per cent of the beets were infected, whereas 11.1 per cent infections were obtained with undiluted extracted beet-root juice which was not centrifuged. According to the summary of table 14 all of the 8 centrifuged preparations used as undiluted controls were infectious while only 2 of 6 preparations not centrifuged used as undiluted controls were infectious. The summary of table 14 shows that 2 of 5 preparations with a dilution of 1:500, 3 of 8 preparations with a dilution of 1:600, and 1 of 8 preparations with each dilution of from 1:700 to 1:1,000 were infectious.

In four additional tests not listed in table 14, dilutions were made at intervals of 100 from 1,000 to 2,000,[5] and at intervals of 1,000 from 1,000 to 10,000, but no infections were obtained with 147 beets that were inoculated.

In Filtered Juice.—The tolerance to dilution of filtered diseased beet-root juice was 1:300. The number of preparations tested and found to be infectious is shown in the summary of table 15.

VIRUS EXTRACT FROM INFECTIVE BEET LEAFHOPPERS

Diluents.—Different diluents were tested with the virus extract from crushed infective beet leafhoppers. The leafhoppers were transferred from infected beets to Mammoth or Alameda sweet corn, which is immune to curly top so that the alimentary canal would not contain unchanged diseased beet juice. One gram of leafhoppers was crushed in a mortar with a pestle in a Schultz mechanical grinder, and then 99 cc of equal parts of steam-extracted beet-root juice and a 5 per cent beet-sugar solution were added. The mixture was centrifuged for 1 hour at 3,500 revolutions per minute and was then used as a stock solution for dilution. The results obtained with different diluents are indicated in table 16.

A comparison of the percentages of beets infected with the virus extract from infective beet leafhoppers diluted with different diluents in table 16, and the number of preparations found to be infectious, as given

[5] During the spring of 1933 a dilution of 1:2,000 was obtained with centrifuged diseased beet-root juice from beets removed from the field.

TABLE 16

INOCULATIONS OF HEALTHY BEET SEEDLINGS WITH CURLY TOP BY MEANS OF NYMPHS FED ON THE VIRUS EXTRACT FROM INFECTIVE BEET LEAFHOPPERS DILUTED WITH DIFFERENT DILUENTS

Preparation No.	Ratio of virus extract of infective leafhoppers to diluent									
	1:100		1:200		1:300		1:400		1:500	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
Diluted with equal parts of steam-extracted beet-root juice and a 5 per cent sugar solution										
1	6	3	6	1	....	....	....	....	....	....
2	3	0	6	2	6	2	9	0	....	....
3	3	1	....	....	....	....	9	3	12	5
4	3	0	....	....	....	....	....	....	9	1
5	6	0	9	0	15	3	3	0	....	....
Total	21	4	21	3	21	5	21	3	21	6
Percentage	....	*19.0*	....	*14.3*	....	*23.8*	....	*14.3*	....	*28.6*
Diluted with 5 per cent sugar solution										
6	6	2	6	3	....	....	....	....	....	....
7	3	3	6	6	6	5	9	1	....	....
8	3	1	....	....	....	....	9	4	12	4
9	9	2	9	1	15	4	3	0	9	0
Total	21	8	21	10	21	9	21	5	21	4
Percentage	....	*38.1*	....	*47.6*	....	*42.9*	....	*23.8*	....	*19.0*
Diluted with distilled water										
10	6	3	6	5	....	....	....	....	....	....
11	3	2	6	5	6	4	9	3	....	....
12	3	2	....	....	....	....	9	7	12	7
13	3	0	....	....	....	....	....	....	6	1
14	6	1	9	2	15	0	3	0	3	0
Total	21	8	21	12	21	4	21	10	21	8
Percentage	....	*38.1*	....	*57.1*	....	*19.0*	....	*47.6*	....	*38.1*

Results summarized according to the number of preparations tested and found infectious

Diluent	Ratio of virus extract of infective leafhoppers to diluent									
	1:100		1:200		1:300		1:400		1:500	
	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious
Steam-extracted beet-root juice and sugar solution	5	2	3	2	2	2	3	1	2	2
Sugar solution	4	4	3	3	2	2	3	2	2	1
Distilled water	5	4	3	3	2	1	3	2	3	2

TABLE 17

Inoculations of Healthy Beet Seedlings with Curly Top by Means of Different Numbers of Previously Noninfective Male Leafhoppers or Nymphs Exposed for Varying Periods to Diluted Virus Extracts from Infective Beet Leafhoppers

Experiment No. 1

Feeding period, hours	Number of insects exposed to each beet	Dilutions of virus extract from infective leafhoppers							
		1:100		1:1,000		1:5,000		1:10,000	
		Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
2	1	5	0	5	0	5	0	5	0
	5	1	0	1	0	1	0	1	0
	10	1	0	1	0	1	0	1	0
	20	1	0	1	0	1	0	1	0
2	1	5	0	5	0	5	0	5	0
	5	1	0	1	0	1	0	1	0
	10	1	1	1	0	1	0	1	0
	20	1	0	1	0	1	0	1	0
4	1	5	1	5	0	5	0	5	0
	5	1	1	1	0	1	0	1	0
	10	1	1	1	0	1	0	1	0
	20	1	1	1	0	1	0	1	0
4	1	5	1	5	0	5	0	5	0
	5	1	0	1	0	1	0	1	0
	10	1	1	1	1	1	0	1	0
	20	1	0	1	0	1	0	1	0
8	1	5	0	5	0	5	0	5	0
	5	1	1	1	0	1	0	1	0
	10	1	0	1	0	1	0	1	0
	20	1	0	1	0	1	0	1	0
8	1	5	1	5	0	5	1	5	0
	5	1	0	1	1	1	0	1	0
	10	1	0	1	0	1	0	1	0
	20	1	1	1	1	1	0	1	1

(Table 17 continued on opposite page)

in the summary in this table, shows that better results were obtained with a 5 per cent solution of beet sugar and with sterile distilled water than with equal parts of steam-extracted beet-root juice and a 5 per cent solution of beet sugar.

Mass Inoculation. — An experiment was conducted to determine whether the time of exposure of varying numbers of leafhoppers to the virus extract from infective beet leafhoppers was a factor in curly-top transmission. Groups of 1, 5, 10, and 20 previously noninfective male leafhoppers were exposed to the feeding solution for periods of 2, 4, and 8 hours, and then each group was transferred to a healthy beet seedling. Various dilutions were used to determine whether single insects were able to transmit curly top by exposure to high dilutions of the virus extract from infective beet leafhoppers. Sterile distilled water was used as a diluent. The results with different dilutions in experiment No. 1 are indicated in table 17.

TABLE 17—*(Concluded)*

Experiment No. 2

Feeding period, hours	Number of insects exposed to each beet	Dilutions of virus extract from infective leafhoppers									
		1:100		1:200		1:300		1:400		1:500	
		Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
2	1	5	0	5	0	5	0	5	0	5	0
	5	1	0	1	0	1	0	1	0	1	0
	10	1	0	1	0	1	0	1	0	1	0
	20	1	0	1	0	1	0	1	0	1	0
4	1	5	0	5	0	5	0	5	0	5	0
	5	1	0	1	0	1	0	1	0	1	1
	10	1	0	1	0	1	0	1	0	1	0
	20	1	1	1	0	1	0	1	0	1	1
6	1	5	0	5	0	5	0	5	0	5	0
	5	1	0	1	0	1	0	1	0	1	0
	10	1	0	1	0	1	0	1	0	1	1
	20	1	0	1	0	1	0	1	0	1	
6	1	5	0	5	2	5	0	5	0	5	1
	5	1	0	1	0	1	0	1	0	1	0
	10	1	0	1	0	1	0	1	0	1	0
	20	1	0	1	0	1	0	1	0	1	0
18	1	5	0	5	0	5	0	5	0	5	0
	5	1	0	1	0	1	0	1	0	1	0
	10	1	0	1	0	1	0	1	0	1	0
	20	1	1	1	0	1	0	1	0	1	0
18	1	5	0	5	0	5	0	5	0	5	0
	5	1	0	1	0	1	0	1	0	1	0
	10	1	0	1	0	1	0	1	0	1	0
	20	1	0	1	0	1	0	1	0	1	0

Since the period of exposure on unfiltered virus extracts was about 6 hours and on filtrates about 18 hours in the experiments reported in this paper, additional tests were made with groups of 1, 5, 10, and 20 male leafhoppers or nymphs exposed to various dilutions of the virus extract from infective beet leafhoppers for periods of 2, 4, 6, and 18 hours, as indicated in experiment No. 2, table 17. The dilutions were made at intervals of 100 from 1:100 to 1:500.

In experiment No. 1, table 17, 3 infections were obtained with single insects exposed to a dilution of 1:100 for periods of 4 or 8 hours, and 1 infection with a dilution of 1:5,000 for 8 hours. Two infections were obtained with groups of 5 insects exposed to a dilution of 1:100 and 1 infection with a dilution of 1:1,000 for 8 hours. Groups of 10 leafhoppers produced 3 infections after an exposure to a dilution of 1:100 for periods of 2 or 4 hours and 1 infection after an exposure to a dilution of 1:1,000 for 4 hours. Two infections with 20 leafhoppers were produced after an exposure to a dilution of 1:100 for 4 or 8 hours and 2 infections with a dilution of 1:1,000 and 1:10,000 for 8 hours.

In experiment 2, table 17, 2 infections were obtained with single insects exposed to a dilution of 1:200 for 6 hours, and 1 infection with a

dilution of 1:500 for 6 hours. One infection was obtained with each group of 5 and 10 insects exposed to a dilution of 1:500 for periods of 4 and 6 hours respectively. Groups of 20 leafhoppers produced 2 infections after exposures to dilutions of 1:100 and 1:500 for 4 hours and 1 infection after an exposure to a dilution of 1:100 for 18 hours.

As shown in table 17, 104 beets were inoculated by varying numbers of insects exposed for a period of 2 hours to various dilutions of the virus extract from infective beet leafhoppers, but only 1 beet, inoculated by 10 males exposed to a dilution of 1:100, became infected. Better results were obtained with groups of 1, 5, 10, and 20 insects exposed for a period of 4, 6, or 8 hours to various dilutions of the virus extract from infective leafhoppers. Eighty beets were inoculated by varying numbers of insects exposed for a period of 18 hours to various dilutions of the virus extract from infective beet leafhoppers, but only 1 beet, inoculated by 20 males exposed to a dilution of 1:100, became infected. During high temperatures the leafhoppers feed continuously and rarely withdraw their mouth parts from the feeding solution. During the night with a lowering of the temperature the insects do not feed as often, and this may explain the small number of infections which were obtained during a feeding period of 18 hours. The insects were fed during the afternoon and night and transferred to healthy beets during the next morning.

The infections with groups of 1, 5, 10, and 20 leafhoppers in the two experiments were as follows: 270 insects tested singly 2.6 per cent; 54 groups of 5 insects or a total of 270, 7.4 per cent; 54 groups of 10 insects or a total of 540, 9.3 per cent; and 54 groups of 20 insects or a total of 1,080, 12.96 per cent.

It may be possible that small quantities of virus repeatedly inoculated by groups of leafhoppers into many parts of a beet low in resistance may multiply and produce the disease. On the other hand, it may be possible that small quantities of virus repeatedly inoculated into the beet is not sufficient to produce infection, and that the minimal infective dose must be present in the leafhopper. If this is the case then the insects tested singly should produce about the same percentage of infection as groups of insects, provided the total number of insects is the same in each test.

DILUTION OF VIRUS FROM INFECTIVE BEET LEAFHOPPERS

The tolerance to dilution of the curly-top virus was determined with the virus extract from crushed infective beet leafhoppers. One gram of infective leafhoppers, or approximately 1,000 specimens, which had completed the nymphal stages on diseased beets were ground with pul-

verized pyrex glass with a pestle in a mortar in a Schultz mechanical grinder. To each gram of crushed leafhoppers, 99 cc of equal parts of autoclaved beet-root juice and a 5 per cent beet-sugar solution was added. The mixture containing the crushed insects was centrifuged for 1 hour at 3,500 revolutions per minute. The virus extract thus obtained was used as a stock solution for dilution. Sterile distilled water was used as a diluent. The results are indicated in table 18.

Centrifuged Virus Extract.—The tolerance to dilution of the virus extract from crushed infective beet leafhoppers was 1 :24,000, as shown in table 18. The summary in table 18 shows that 1 of 6 preparations were infectious at a dilution of 1 :22,000 and 3 of 6 preparations at 1 :20,000. With a dilution of 1 :10,000, all of the 5 preparations tested were infectious, but only 7 of 42 beets were infected in 14 feeding experiments with 5 different preparations. In two additional tests not listed in table 18, dilutions were made at 1 :45,000 and 1 :50,000 but no infections were obtained with 42 beets.

Apparently a higher concentration of the virus occurred in the infective beet leafhoppers which completed the nymphal instars on diseased beets than in diseased beet roots, if we are justified in making the comparison. One gram of beet leafhoppers was crushed in 99 cc of feeding solution, and the mixture was centrifuged, throwing down the chitin and probably most of the protoplasm. Diseased beet-root juice was centrifuged, throwing down the cellulose cell walls and probably most of the protoplasm. One cc of the virus extract from infective beet leafhoppers and 1 cc of centrifuged diseased beet-root juice was used in the lower dilutions and subdilutions were made in the higher dilutions. The dilution medium for both virus extracts from infective beet leafhoppers and diseased beet-root juice was sterile distilled water. The specific gravity of the stock solutions used for dilution with the virus extracts from infective beet leafhoppers and with the diseased beet-root juice was not determined.

It may be argued that the tolerance to dilution of the curly-top virus is not comparable to the tolerance to dilution obtained by mechanical inoculation of mosaic viruses since a small amount of virus may multiply or increase within the body of the beet leafhopper. A number of scientists have suggested that a multiplication of the virus occurs within the bodies of insects which transmit virus diseases of the yellows group. This theory was based on the fact that some insects retained the infective power through life, while others lost it quickly. The literature fails to show that a single experiment has been performed to prove or disprove the theory that the virus multiplies or increases within the body of the insect.

TABLE 18

INOCULATIONS OF HEALTHY BEET SEEDLINGS WITH CURLY TOP BY MEANS OF NYMPHS FED ON DILUTED VIRUS EXTRACT FROM INFECTIVE BEET LEAFHOPPERS

Dilutions of virus extract from infective leafhoppers

Preparation No.	1:100		1:200		1:300		1:400		1:500		1:600		1:700		1:800		1:900		1:1,000	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
1	6	3	6	5	....	....	....	....	6	1	....	....	....	....	....	....	....	....	....	....
2	3	2	6	6	6	4	9	3	12	7	....	....	....	....	....	....	....	....	....	....
3	3	2	....	....	6	4	9	7	....	....	....	....	....	....	....	....	....	....	....	....
4	3	0	3	1	....	....	3	0	3	2	....	....	....	....	....	....	....	....	....	....
5	3	1	3	2	....	....	3	2	3	2	6	0	9	0	....	....	6	1	12	1
6	6	2	....	....	....	....	....	....	....	....	3	0	3	2	....	....	....	....	3	3
7	6	3	....	....	....	....	....	....	....	....	3	3	3	1	6	3	6	4	6	2
8	6	0	....	....	....	....	....	....	....	....	3	0	3	0	3	2	3	0	3	0
9	3	2	....	....	....	....	....	....	....	....	6	2	6	1	6	2	6	0	6	1
Total	39	15	18	14	12	8	24	12	24	12	21	5	24	4	15	7	21	5	30	7
Percentage	....	*38.5*	....	*77.8*	....	*66.7*	....	*50.0*	....	*50.0*	....	*23.8*	....	*16.7*	....	*46.7*	....	*23.8*	....	*23.3*

Dilutions of virus extract from infective leafhoppers

	1:100		1:1,100		1:1,200		1:1,300		1:1,400		1:1,500		1:1,600		1:1,700		1:1,800		1:1,900	
10	3	2	3	0	3	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
11	3	2	3	1	3	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
12	6	0	12	3	12	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
13	6	3	6	3	6	5	....	....	....	....	....	....	....	....	....	....	....	....	....	....
14	3	1	....	....	....	....	6	4	6	0	6	2	6	3	6	5	6	5	6	1
Total	21	8	24	7	24	5	6	4	6	0	6	2	6	3	6	5	6	5	6	1
Percentage	....	*38.1*	....	*29.2*	....	*20.8*	....	*66.7*	....	*0.0*	....	*33.3*	....	*50.0*	....	*83.3*	....	*83.3*	....	*16.7*

Dilutions of virus extract from infective leafhoppers

	1:100		1:2,000		1:3,000		1:4,000		1:5,000		1:6,000		1:7,000		1:8,000		1:9,000		1:10,000	
15	3	2	6	3	....	....	....	....	....	....	....	....	....	....	....	....	....	....	12	2
16	3	2	3	2	....	....	....	....	....	....	....	....	....	....	....	....	....	....	12	1
17	3	2	3	1	3	0	3	0	3	1	3	3	3	0	3	0	3	1	6	2
18	3	2	....	....	6	1	6	1	6	1	3	0	3	1	3	2	3	1	6	1
19	3	0	3	0	....	....	....	....	....	....	3	0	3	1	3	0	3	0	6	4
Total	15	8	15	6	9	1	9	1	9	2	9	3	9	2	9	2	9	2	42	7
Percentage	....	*53.3*	....	*40.0*	....	*11.1*	....	*11.1*	....	*22.2*	....	*33.3*	....	*22.2*	....	*22.2*	....	*22.2*	....	*16.7*

TABLE 18—*(Concluded)*

Dilutions of virus extract from infective leafhoppers

	1:100		1:11,000		1:12,000		1:13,000		1:14,000		1:15,000		1:16,000		1:17,000		1:18,000		1:19,000	
20	3	1	3	0	3	0	3	0	3	1	3	0	3	0	3	0	3	0	3	0
21	3	3	3	0	3	0	3	0	3	0	6	0	3	0	3	1	3	0	3	0
22	3	1	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0
23	3	1	3	0	3	0	3	0	3	0	3	0	3	1	3	0	3	0	3	0
24	3	1	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0
25	3	3	3	1	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0
26	3	1	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0
27	3	1	3	0	3	0	3	0	3	0	15	1	3	0	3	0	3	0	3	0
Total	24	12	24	1	24	0	24	0	24	1	39	1	24	1	24	1	24	0	24	0
Percentage	....	*50.0*	....	*4.2*	....	*0.0*	....	*0.0*	....	*4.2*	..	*2.6*	....	*4.2*	....	*4.2*	....	*0.0*	....	*0.0*

Dilutions of virus extract from infective leafhoppers

	1:100		1:20,000		1:21,000		1:22,000		1:23,000		1:24.000		1:25,000		1:30,000		1:35,000		1:40,000	
28	9	3	6	0	3	0	3	0	3	0	3	0	6	0	6	0	6	0	6	0
29	3	2	3	1	3	0	3	1	3	0	3	0	6	0	6	0	6	0	6	0
30	3	2	3	1	3	0	3	0	3	0	3	0	6	0	3	0	3	0	3	0
31	3	1	3	0	3	0	3	0	3	0	3	1	6	0	3	0	3	0	3	0
32	3	3	6	1	3	0	3	0	3	0	3	0	6	0	3	0	3	0	3	0
33	3	3	6	0	3	0	3	0	3	0	3	0	12	0	3	0	3	0	3	0
Total	24	14	27	3	18	0	18	1	18	0	18	1	42	0	24	0	24	0	24	0
Percentage	....	*58.3*	....	*11.1*	....	*0.0*	....	*5.6*	....	*0.0*	....	*5.6*	....	*0.0*	....	*0.0*	....	*0.0*	....	*0.0*

Results summarized according to the number of preparations tested and found infectious

Dilution	Tested	Infectious	Dilution	Tested	Infectious	Dilution	Tested	Infectious	Dilution	Tested	Infectious	Dilution	Tested	Infectious
1:100	9	7	1:100	5	4	1:100	5	4	1:100	8	8	1:100	6	6
1:200	4	4	1:1,100	4	3	1:2,000	4	3	1:11,000	8	1	1:20,000	6	3
1:300	2	2	1:1,200	4	1	1:3,000	2	1	1:12,000	8	0	1:21,000	6	0
1:400	4	3	1:1,300	1	1	1:4,000	2	1	1:13,000	8	0	1:22,000	6	1
1:500	4	4	1:1,400	1	0	1:5,000	2	2	1:14,000	8	1	1:23,000	6	0
1:600	5	2	1:1,500	1	1	1:6,000	3	1	1:15,000	8	1	1:24,000	6	1
1:700	5	3	1:1,600	1	1	1:7,000	3	2	1:16,000	8	1	1:25,000	6	0
1:800	3	3	1:1,700	1	1	1:8,000	3	1	1:17,000	8	1	1:30,000	6	0
1:900	4	2	1:1,800	1	1	1:9,000	3	2	1:18,000	8	0	1:35,000	6	0
1:1,000	5	4	1:1,900	1	1	1:10,000	5	5	1:19,000	8	0	1:40,000	6	0

TABLE 19

THERMAL DEATH POINT OF CURLY-TOP VIRUS IN EXTRACTED, CENTRIFUGED, AND FILTERED DISEASED BEET-ROOT JUICE

Preparation No.	Unheated control		55° C		60° C		65° C		70° C		75° C		80° C		85° C	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
Extracted diseased beet-root juice																
1	3	1	3	1	3	0	3	0	6	0	6	0	3	0	3	0
2	3	0	3	0	3	1	3	0	6	0	6	0	3	0	3	0
3	3	0	3	3	3	0	6	0	6	0	6	0	3	0	3	0
4	3	3	....	....	3	3	3	2	6	0	6	0	....	....	3	0
5	3	3	....	....	6	4	6	1	6	1	6	0	....	....	3	0
6	3	2	....	....	....	....	6	3	6	3	18	0	....	....	3	0
Total	18	9	9	4	18	8	27	6	36	4	48	0	9	0	18	0
Percentage	....	*50.0*	....	*44.4*	....	*44.4*	....	*22.2*	....	*11.1*	....	*0.0*	....	*0.0*	....	*0.0*
Centrifuged diseased beet-root juice																
7	3	3	9	7	9	9	....	....	....	....	9	1	9	0	3	0
8	3	3	9	9	9	4	9	6	9	1	....	....	....	....	3	0
9	3	2	9	7	9	4	....	....	12	1	9	0	....	....	3	0
10	3	3	....	....	9	6	....	....	6	0	9	0	....	....	3	0
11	3	2	....	....	....	....	9	3	....	....	....	....	9	0	3	0
12	3	3	....	....	....	....	9	8	....	....	9	0	9	0	3	0
Total	18	16	27	23	36	23	27	17	27	2	36	1	27	0	18	0
Percentage	....	*88.9*	....	*85.2*	....	*63.9*	....	*63.0*	....	*7.4*	....	*2.8*	....	*0.0*	....	*0.0*
Filtrate prepared from diseased beet-root juice																
13	3	2	....	....	....	....	9	2	9	0	9	0	3	0	6	0
14	3	3	....	....	....	....	6	6	9	1	9	0	3	0	6	0
15	3	1	....	....	....	....	6	0	6	0	6	0	3	0	3	0
16	3	2	....	....	....	....	....	....	....	....	6	0	3	0	3	0
Total	12	8	....	....	....	....	21	8	24	1	30	0	12	0	18	0
Percentage	....	*66.7*	....	....	....	....	....	*38.1*	....	*4.2*	....	*0.0*	....	*0.0*	....	*0.0*

Results summarized according to the number of preparations tested and found infectious

Source of preparation	Unheated control		55° C		60° C		65° C		70° C		75° C		80° C		85° C	
	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious
Extracted root juice	6	4	3	2	5	3	6	3	6	2	6	0	3	0	6	0
Centrifuged root juice	6	6	3	3	4	4	3	3	3	2	4	1	3	0	6	0
Filtered root juice	4	4	....	....	....	....	3	2	3	1	4	0	4	0	4	0

THERMAL DEATH POINT OF VIRUS

The thermal death point of the curly-top virus was determined in extracted, centrifuged, and filtered beet-root juice. The diseased juice was poured into pyrex glass tubing sealed at one end. The average thickness of the wall in the tubing was about 0.6 mm and the inside diameter was 8 mm. Ten cc of root juice was placed in each tube and then the open end was sealed by flame. A sealed tube was approximately three-fourths filled with juice. The tubes were submerged in a water bath controlled by an electric thermostat. A clinical thermometer was put into one tube containing the root juice, and after a number of tests it was found that it required about 1 minute for the heat to penetrate the glass tubing, and bring the temperature of the contents to that of the constant-temperature water bath. The time of exposure in the water bath was 11 minutes, 1 minute being allowed for lag. In later tests, 10 cc of beet-root juice was poured in thin-walled test tubes plugged with cotton. A submersion thermometer with 0.5° C graduations was used in the water bath. The time of exposure in the water bath was 10 minutes. After exposure to the desired temperature the tubes were cooled rapidly in running tap water. Unheated controls were always used. Determinations were made only at 5° C intervals. The results are shown in table 19.

According to table 19 the curly-top virus was inactivated in 10-minute exposure by a temperature of 75° C in the extracted and filtered diseased beet-root juice and 80° C in the centrifuged root juice. The summary in table 19 shows that of 14 preparations of extracted, centrifuged, and filtered diseased beet-root juice heated at 75° C only 1 was found to be infectious. No infections were obtained with 10 preparations heated at 80° C and 16 preparations heated at 85° C. When beet-root juice was exposed to temperatures ranging from 70° to 85° C, a coagulation of the juice sometimes occurred which may have protected the virus.

The thermal death point of the curly-top virus from crushed infective beet leafhoppers was also determined, using the same methods as were used with diseased beet-root juice. The results are indicated in table 20.

It is evident from table 20 that no infections were obtained with the virus extract, centrifuged virus extract, and filtrate prepared from the virus extract of infective beet leafhoppers heated at 80° C in 10-minute exposure. The summary in table 20 shows that of 13 preparations heated at 75° C, 4 were found to be infectious.

TABLE 20

THERMAL DEATH POINT OF VIRUS EXTRACT, CENTRIFUGED VIRUS EXTRACT, AND FILTRATE PREPARED FROM VIRUS EXTRACT OF CRUSHED INFECTIVE BEET LEAFHOPPERS

Preparation No.	Unheated control		55° C		60° C		65° C		70° C		75° C		80° C	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Beets inoculated	Beets infected
Virus extract from infective leafhoppers														
1	3	0	3	0	3	1	....	....	....	....	6	0	6	0
2	3	0	6	0	6	1	....	....	....	....	6	0	6	0
3	3	1	....	....	6	5	6	3	6	6	6	0	6	0
4	3	1	9	1	....	....	6	1	6	4	6	1	....	....
5	6	1	12	0	....	....	6	0	6	0	6	0	....	....
6	9	0	9	1	9	0	9	1	9	0	9	0	9	0
Total	27	3	39	2	24	7	27	5	27	10	39	1	27	0
Percentage	....	*11.1*	....	*5.1*	....	*29.2*	....	*18.5*	....	*37.0*	....	*2.6*	....	*0.0*
Centrifuged virus extract from infective leafhoppers														
7	6	3	9	0	9	3	9	0	9	0	9	3	12	0
8	6	3	9	2	9	1	9	0	9	0	9	0	9	0
9	3	1	9	3	9	0	9	2	9	3	9	1	9	0
Total	15	7	27	5	27	4	27	2	27	3	27	4	30	0
Percentage	....	*46.7*	....	*18.5*	....	*14.8*	....	*7.4*	....	*11.1*	....	*14.8*	....	*0.0*
Filtrate prepared from virus extract of infective leafhoppers														
10	3	3	9	3	9	0	....	....	....	....	....	....	....	....
11	3	0	....	....	....	....	6	1	6	0	6	0	....	....
12	3	0	....	....	6	2	6	0	6	0	6	0	6	0
13	3	2	....	....	....	....	6	1	6	0	6	0	6	0
14	3	2	....	....	....	....	....	....	....	....	12	1	6	0
Total	15	7	9	3	15	2	18	2	18	0	30	1	18	0
Percentage	....	*46.7*	....	*33.3*	....	*13.3*	....	*11.1*	....	*0.0*	....	*3.3*	....	*0.0*

Results summarized according to number of preparations tested and found infectious

Type of preparation	Unheated control		55° C		60° C		65° C		70° C		75° C		80° C	
	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious	Tested	Infectious
Extracted	6	3	5	2	4	3	4	3	4	2	6	1	4	0
Centrifuged	3	3	3	2	3	2	3	1	3	1	3	2	3	0
Filtered	5	3	1	1	2	1	3	2	3	0	4	1	3	0

EFFECT OF FREEZING BEET-ROOT EXTRACT ON VIRUS

The filtrate prepared from undiluted diseased beet-root juice was kept in cold storage at a temperature of 0° C and –18° C. Freezing did not inactivate the curly-top virus. The number of infections obtained by exposing previously noninfective nymphs to the filtrate weekly for a period of 8 weeks and then transferring the insects to 3 healthy beet seedlings in each test is shown in table 21.

TABLE 21

INOCULATIONS OF HEALTHY BEET SEEDLINGS BY MEANS OF NYMPHS FED ON FILTRATE PREPARED FROM DISEASED BEET-ROOT JUICE KEPT IN COLD STORAGE

Age of filtrate, weeks	Temperature in cold storage			
	0° C		—18° C	
	Beets inoculated	Beets infected	Beets inoculated	Beets infected
1	3	2	3	0
2	3	3	3	3
3	3	2	3	2
4	3	1	3	3
5	3	1	3	3
6	3	1	3	1
7	3	2	3	2
8	3	0	3	2

In another test freezing did not cause an inactivation of the virus at a temperature of –18° C at the end of 11 months and 8 days.

A COMPARISON OF SOME PROPERTIES OF CERTAIN MOSAIC VIRUSES WITH THOSE OF THE CURLY-TOP VIRUS

It is not the purpose of this discussion to review all of the literature on the properties of mosaic viruses but simply to compare certain properties of mosaic viruses with the curly-top viruses of the yellows group.

Aging of Viruses in Extracted Juices.—There is a considerable difference in the longevity of various mosaic viruses in juices extracted from diseased plants. Tobacco mosaic virus has been found by all scientists to remain infectious for a considerable period after extraction from diseased plants, as indicated in table 22.

TABLE 22

COMPARISON OF SOME PROPERTIES OF VIRUSES OF MOSAIC GROUP WITH THOSE OF CURLY TOP OF YELLOWS GROUP

Virus	Aging of viruses in extracted juice*	Tolerance to dilution	Thermal death point, 10-minute exposure	Filterability
Mosaic viruses				
Tobacco mosaic (tobacco virus 1)	+3 yrs.[63]	1:1,000,000[2,47,22]	93° C 5 minutes[3]	Filterable[1,4,5,12,13,26,27,34]
	+2 yrs.[29]		90° C[3]	
	+4 or 5 mos., −15 mos.[1]		90° C[29]	
			95° C[32]	
Tomato mosaic identical with tobacco mosaic	+138 days[63]	1:10,000[63]	85° to 90° C[63]	Filterable[6,63]
			88° C[6]	
Yellow tobacco mosaic (tobacco virus 6)	+3 mos.[29]	1:1,000,000[47]	90° C[29]	
Tomato mosaic identical with yellow tobacco mosaic		1:10,000[56]	90° C[56]	
Speckled tobacco mosaic (tobacco virus 2)	+3 mos.[29]		90° C[29]	
Medium tobacco mosaic (tobacco virus 7)	+3 mos.[29]		90° C[29]	
Tomato-stem necrosis (tobacco virus 9)	+3 mos.[29]		90° C[29]	
Bleaching mosaic (tobacco virus 8)	−3 days[29]		75° C[29]	
Ringspot of tobacco (tobacco virus 5)	−14 days[29]	1:10,000[47]	70° C[29,32]	Not filterable[45]
	−6 days[32]			
Mild mosaic (tobacco virus 3	−6 days[29]		60° C[29]	

TABLE 22—*(Concluded)*

Virus	Aging of viruses in extracted juice*	Tolerance to dilution	Thermal death point, 10-minute exposure	Filterability
Mosaic viruses				
Spot necrosis 2 viruses	−14 days[29]	1:10,000[47]	70° C[29]	
Spot-necrosis form	−20 days[32]		60° C[32]	
Potato rugose mosaic[33]	+6 hrs., −24 hrs.[30]	1:10[30]	Close to 43° C[30]	
	−24 to 48 hrs.[31]	1:100[31]	60° to 65° C[31]	
Mottle form	+20 days[32]		70° C[32]	
Petunia mosaic (petunia virus)		1:10,000[47]		
Cucumber mosaic (cucumber virus 1)	−3 to 5 days[15]	1:10,000[15]	75° C[15]	Filterable[14,15,28,63]
	−3 days[29], +3 days[32]	1:100,000[32]	60° C[29], 70° C[32]	
	−2 days[63]			
Bean mosaic	−20 to 24 hrs.[19]	1:1,000[19]	44° to 56° C[19]	Not filterable[19]
Dock mosaic	+14 days[24]	1:100[24]	80° C[24]	Not filterable[24]
Crinkle mosaic of potatoes	−24 to 48 hrs.[31]	1:10[31]	43° to 45° C[31]	
Mild mosaic of potatoes	−2 to 4 hrs.[31]	1:100[31]	40° to 45° C[31]	
Leafroll mosaic of potatoes	−24 to 48 hrs.[31]	1:200[31]	70° to 75° C[31]	
Yellows virus				
Curly top of sugar beets	−72 hrs.	1:1,000	80° C	Filterable

* The plus sign (+) indicates that infections were obtained, and the minus sign (−) shows that the virus was inactivated.

Priode[45] found that the length of time during which the virus of ringspot disease of tobacco retains its virulence in expressed juice varies inversely with the temperature at which the juice is stored. The virus held at –5° C retained its virulence over a period of 85 days during which the experiment was in progress. A sample held at 0° C lost its virulence after about 3 weeks, one held at 10° C after 12 days, and one held at 5° C after 20 days.

Holmes[25] in working on the effect of aging on the tobacco mosaic virus found that at room temperatures the virus was reduced to 2 per cent of its original strength in a month.

Doolittle[15] found that the expressed juices of mosaic plants of several species of cucurbits were never infectious for more than 3 to 5 days and in most cases had lost their virulence within 24 to 48 hours.

Fajardo[19] found that the resistance to aging of the bean mosaic virus was 20 to 24 hours.

A rapid inactivation of the curly-top virus occurred after extracted beet-root juice was exposed to the air at room temperature for a period of 40 to 48 hours.

Resistance of Virus to Drying in Plant Tissues.—It is well known that the infective principle of common tobacco mosaic is retained in dried leaves for long periods of time. Valleau and Johnson[61, 62] have shown that old natural leaf tobacco seems to carry the virus in as virulent a form as fresh tobacco, and the disease has been produced by inoculations with samples of tobacco 5, 16, 17, 18, 20, 30, and 31 years old. Infections with preparations from dried mosaic plants have been obtained by Chapman[12] after 3 years, Beijerinck[4] after 2 years, and Allard[1] after 18 months.

Doolittle[15] found that the leaves of cucumber mosaic plants when dried at room temperatures for periods of 10 days to 1 year failed to produce the disease.

Grainger and Cockerham[24] allowed dock mosaic leaves to dry in the air for a period of 21 days and obtained infections with the inoculum prepared from them.

Fajardo[19] obtained infections with bean mosaic virus from seedlings that had been allowed to dry at room temperatures for 48 hours, but no infections were secured from plants after drying 72 hours.

The curly-top virus was inactivated in the pulp of diseased beet roots slowly dried in the greenhouse for a period of 7 weeks and in the headhouse for a period of 5 and 7 weeks, and also in diseased beet roots thoroughly dried in the greenhouse.

Tolerance to Dilution.—Allard[2] showed that the virus of tobacco mosaic could be diluted to 1:1,000 without reducing virulence. He also

made a number of successful inoculations with the dilution of 1:10,000 and 1:1,000,000 although these higher dilutions rarely gave infections. Walker[63] obtained infections with a dilution of 1:10,000 with the virus of tomato mosaic, which is apparently identical with tobacco mosaic.

Doolittle[15] reported similar results with cucumber mosaic. Dilutions of 1:1,000 were potent as undiluted solutions, but while infections may result from those of 1:10,000 they have never taken place at higher dilutions.

Samuel[47] has shown by means of dilution tests that a light rubbing with the virus in which no visible wound is produced on the leaf, is a more effective method of mechanical inoculation than scratching with a needle in the case of five viruses diluted as follows: tobacco mosaic 1:1,000,000; yellow tobacco mosaic 1:1,000,000; spot-necrosis of tobacco 1:10,000; ringspot of tobacco 1:10,000; and petunia mosaic 1:10,000.

Johnson and Grant[32] obtained a few infections using the rubbing method of inoculation with a dilution of 1:100,000 with the virus of cucumber mosaic.

According to Johnson[31] the following potato viruses were for the most part relatively intolerant to dilution: crinkle mosaic 1:10; rugose mosaic 1:100; mild mosaic 1:100; and leafroll mosaic 1:200.

Grainger and Cockerham[24] found that the tolerance to dilution of the virus of dock mosaic was 1:100.

Fajardo[19] obtained a low percentage of infections with the bean mosaic virus with a dilution of 1:1,000.

The tolerance to dilution of the curly-top virus in beet-root juice was 1:1,000, corresponding to the dilution magnitude of the bean-mosaic virus.

Thermal Death Point.—The thermal death points of mosaic viruses vary from 40°–45° C in mild mosaic of potatoes to 90°–95° C in tobacco mosaic in 10-minute exposure, as indicated in table 22. Exposure of the virus of tobacco mosaic to 80° C as long as 20 days did not completely destroy it, but 83°–84° C for 24 hours destroyed it.[42]

McKinney[39] found that the thermal death point was lowered approximately 6° C when juice of mosaic tobacco plants was diluted 1:100 in 10 minutes' exposure. According to table 22 the virus of dock mosaic was inactivated when the extract was heated at 80° C for 10 minutes; this corresponds with the thermal death point of the curly-top virus.

Effect of Freezing on Virus.—Allard[3] demonstrated that fresh sap extracted from tobacco mosaic plants frozen in liquid air at a temperature of –180° C for 15 minutes at –12° C for 4 hours, and exposed out-of-doors during the entire winter and allowed to freeze and thaw repeatedly still retained its infectious properties.

Holmes[25] found that tobacco mosaic virus stored below the freezing point lost 85 per cent of its original strength after 1½ months.

Brewer, *et al.*[6] found that purified virus suspensions of tomato mosaic stored in a refrigerator at 2.22–4.44° C proved to be infectious after 6 to 20 months.

Fajardo[19] found that freezing undiluted, freshly expressed juice of bean mosaic plants at temperatures of –7° to –10° C did not affect the viability of the virus.

Doolittle[15] reported that low temperatures (specific data not given) have only a slight effect in prolonging the power of infection of the cucumber mosaic virus.

The filtrate prepared from undiluted diseased beet-root juice was kept in cold storage at –18° C. Freezing at this temperature did not inactivate the curly-top virus after 11 months and 8 days.

Filterability.—It has been shown by Iwanowski,[26, 27] Beijerinck,[4, 5] Konig,[34] Allard,[1] Clinton,[13] and Chapman[12] that the virus of tobacco mosaic is capable of passing through Berkefeld or Chamberland filters, but Iwanowski[27] found that only the first portion of the Chamberland filtrate was infectious. According to Allard[3] "the infective principle of tobacco mosaic is retained by the Livingston atmometer porous cup used as a filter, and also by powdered talc;" but Duggar and Karrer[16] readily obtained infections with infective juice passed through a Livingston spherical atmometer cup with pores noticeably finer than the average of these cups. Chapman[12] found that the juice of tobacco mosaic was still infectious after passing through a fine Berkefeld (W) candle and a Kitasta filter.

Walker[63] found that the virus of tomato mosaic, which is apparently identical with tobacco mosaic, is capable of passing through all grades of Berkefeld filters with little loss of infective power, but filtrates from the Chamberland filters gave a low percentage of infection.

Brewer, *et al.*[6] found that purified virus suspensions of tomato mosaic were still active after passage through Pasteur-Chamberland F filters and 1½ per cent Schleicher and Shüll collodion filters, but lost their virulence after passage through an atmometer cylinder, a Pasteur-Chamberland B filter, Schleicher and Shüll 3, 4½, 6, or 7½ per cent collodion filters, or 2, 3, or 5 per cent collodion filters precipitated from solution in equal parts of alcohol and ether. The wash water from the upper surface of used collodion filters was infectious.

Doolittle[14, 15] and Jagger[28] found that cucumber mosaic virus passes through Berkefeld filters but according to Doolittle[15] will not pass through all grades of Chamberland filters. Walker[63] found that

infections occurred after inoculation with the filtrate passed through the fine Berkefeld (W) filter but the filtrates from Chamberland filters gave no infections.

The curly-top virus passed through all grades of Berkefeld (V, N, W), Mandler (preliminary, regular, and fine) candles with reduced pressure, and Chamberland filters (L1, L3, L5, L7, L9, L11, and L13).

A comparison of some of the properties of viruses of the mosaic group with curly top of the yellows group indicates that curly top is caused by a specific virus distinct from viruses that have been similarly studied, although these properties lie within the range of the mosaic viruses. The properties of the curly-top virus so far investigated do not show any marked differences from those of many mosaic viruses.

SUMMARY

Nymphs after feeding on filtered and unfiltered juices extracted from the blades, petioles, and blades and petioles combined, of beet seedlings experimentally infected with curly top in the greenhouse, failed to transmit the virus to 214 healthy beets.

Infections were obtained with the juices from the blades, petioles, and blades and petioles combined, of diseased beet seedlings, extracted below the surface of autoclaved beet-root juice and a beet-sugar solution, or in a beet-sugar solution without the root juice. The results seem to indicate that oxidation was a factor in the inactivation of the virus in the first attempt.

Infections were obtained with the juices extracted from the blades and beet roots of large diseased beets removed from the field. No infections were obtained with the juices extracted from the petioles, or blades and petioles combined.

The virus can be more readily transmitted by previously noninfective nymphs exposed to centrifuged and supercentrifuged beet-root juice than by those exposed to similarly treated leaf juice; but no marked difference in the results was obtained with centrifuged and supercentrifuged beet-root juice.

Extracts from diseased leaves and beet roots were diluted with various diluents and then centrifuged, but again the preparations from beet roots gave the best results.

An inactivation of the virus occurred after extracted, centrifuged, and supercentrifuged diseased beet-root juice was exposed to the air at room temperature for a period of 72 hours. The longevity of the

curly-top virus in the filtrate prepared from diseased beet-root juice under aerobic conditions was 8 days. Infections were obtained with the partially anaerobic filtrate prepared from supercentrifuged diseased beet-root juice at the end of 5 weeks. In the filtrate prepared from diseased beet-root juice adjusted to pH 5.0 and pH 6.0 and kept in an anaerobic jar, the virus was recovered after 100 days, the full length of time this experiment was in progress. With the same juice adjusted to pH 3.5, the virus was apparently inactivated the first time that it was tested at the end of 7 days.

Attempts to cultivate the curly-top virus in a feeding solution under anaerobic conditions were failures.

The curly-top virus was inactivated in the pulp of diseased beet roots slowly dried in the greenhouse for a period of 5 weeks and in the headhouse for 7 weeks, also in diseased beet roots dried in the greenhouse for 7 weeks and in dried infective beet leafhoppers.

The virus was inactivated in beet-root juice diluted with centrifuged juice extracted from Alameda or Mammoth sweet-corn plants (immune to curly top) at the rates of 1:50, 1:100, and 1:200, after periods of 2, 4, and 6 hours.

There was no evidence to show that sedimentation of the virus occurred by supercentrifuging beet-root juice three times. There was no increase in the number of infections with the supercentrifuged liquid prepared from the gummy residue resuspended in distilled water. No infections were obtained after the first day with the filtrate containing a mixture of supercentrifuged liquid prepared from the gummy residue and aluminum gel.

The tolerance to dilution of centrifuged diseased beet-root juice was 1:1,000 and was obtained with 49 small beets removed from the field during the spring. The tolerance to dilution of the virus extract from infective beet leafhoppers was 1:24,000.

The thermal death point of the curly-top virus in beet-root juice and the virus extract prepared from infective beet leafhoppers was 80° C in 10-minute exposures.

Freezing filtered beet-root juice kept in cold storage at –18° C did not inactivate the curly-top virus after 11 months and 8 days.

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